Stochastic Regulation of Estrogen Receptor Mediated Transcription

Elizabeth Jones[1], Valeria Berno, Michael Mancini[2], and Marek Kimmel[3]

***Short Abstract* — Gene transcription is a tightly regulated process. Studies in our lab, and in other labs, indicate a stochastic element to the regulation. Our lab has a constructed cell line with an integrated multicopy prolactin promoter array which is easily visualized by GFP-tagged proteins, immunofluorescence, and mRNA FISH. With certain ligand treatements the mRNA FISH intensity fluctuates at the array over time indicating additional intrinsic or extrinsic activation. By quantitatively measuring the intensity of different labeled proteins and developing a mathematical model incorporating a multistep activation process we will have a better systems biology understanding of estrogen receptor (ER) mediated transcription.**

I. Introduction

Regulation of gene transcription is a major contributor of cell diversity by affecting the response and adaptation of the cell to its environment. Moreover, perturbation of the gene regulation plays a key role in the development of numerous diseases, including many cancers. Therefore, understanding the regulation of transcription will be useful in developing treatments for diseases caused by the deregulation of transcription. Due to technological and analytical advances mechanistic understanding of regulation of transcription at the single cell level is an area of expanding research. Transcription is a complex and multistep process that can be regulated at many levels, including transcription factors binding to promoter DNA sequences, interactions of accessory factors, chromatin remodeling, RNA polymerase II recruitment, and mRNA synthesis and processing. Our laboratory has developed quantitative kinetic measurement of many of these transcriptional mechanisms using single cell-based nuclear receptor driven-gene expression as a model system.

II. Biological System

The main objective of this research is to define the dynamic, cooperative, and cyclic nature of gene expression, describing cell to cell diversity and transcriptional bursts. These studies will be performed using a PRL-HeLa cell line which contains a multicopy locus of the prolactin gene and allows for direct visualization of GFP-estrogen receptors (GFP-ER) in living or fixed cells, and quantification of transcription by visualizing report gene mRNA [1]. The PRL array contains multiple ER binding elements. These elements control the expression of a fluorescent reporter protein, dsRed2skl. The promoter locus is easily visualized by microscopy via the accumulation GFP-ER, histone markers, or several transcription-related proteins. The array is also hormonally responsive in terms of transcriptional activity, visible targeting by ER, coactivator recruitment, and reversible large changes to the size and shape of the locus (e.g. large-scale chromatin remodeling). Reporter gene activity is readily quantified by fluorescent in situ hybridization (FISH). Time course FISH studies reveal the amount of transcript distinct cycling of mRNA accumulation while array size remains constant.

Our PRL-HeLa model indicates variable production of mRNA from the integrated multicopy promoter array [2], and provides a unique single cell based opportunity to investigate the role of stochasticity in gene regulation. Experimental approaches are in development that will assess the degree of stochasticity involved in the regulation of the reporter gene. For example, the amount of reporter gene mRNA will be correlated to the quantitative (and automated) measurement of GFP-ER and coactivator(s) with the array over time, and during agonist or antagonist treatments (e.g., estradiol vs. tamoxifen). This approach will lead us to measure possible sources of intrinsic and extrinsic regulation of the fluctuation in transcription.

III. Mathematical Model

I am developing a stochastic mathematical model that describes the activation of a PRL-promoter as a Markov process. It is modeled as a two-step activation process, incorporating both extrinsic and intrinsic activation modeled as stochastic events. By defining the fluctuations of transcription rates and the stochasticity of mRNA production we will add to our general appreciation of the complex transcriptional control mechanisms, and improve our systems biology understanding of gene regulation in development and disease.

[1]Program for Structural and Computational Biology and Molecular Biophysics, Baylor College of Medicine, Houston, TX. E-mail: edjones@bcm.edu

[2]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX. E-mail: mancini@bcm.edu

[3]Department of Statistics, Rice University, Houston, TX. Email: kimmel@rice.edu

References

[1] Sharp ZD, MG Mancini, CA Hinojos, et al. (2006) Estrogen-receptor-α exchange and chromatin dynamics are ligand- and domain-dependent. *J Cell Sci* 119:4365.

[2] Unpublished work by Berno V